Rho GTPases: Biochemistry and Biology

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Abstract

Approximately one percent of the human genome encodes pro-
teins that either regulate or are regulated by direct interaction with
members of the Rho family of small GTPases. Through a series
of complex biochemical networks, these highly conserved molecu-
lar switches control some of the most fundamental processes of cell
biology common to all eukaryotes, including morphogenesis, polar-
ity, movement, and cell division. In the first part of this review, we
present the best characterized of these biochemical pathways; in the
second part, we attempt to integrate these molecular details into a
biological context.

Contents

INTRODUCTION

Rho GTPases constitute a distinct family within the superfamily of Ras-related small GTPases and are found in all eukaryotic cells. Twenty-two mammalian genes encoding Rho GTPases have been described—three Rho isoforms A, B, and C; three Rac isoforms 1, 2, and 3; Cdc42, RhoD, Rnd1, Rnd2, RhoE/Rnd3, RhoG, TC10, and TCL; RhoH/TTF; Chp and Wrch-1; Rif, RhoBTB1, and 2; and Miro-1 and 2 (Aspenstrom et al. 2004). The yeast *Saccharomyces cerevisiae* has 5 Rho proteins (Rho 1, 2, 3, and 4 and Cdc42), whereas *Caenorhabditis elegans* and *Drosophila melanogaster* are predicted to have 10 and 11, respectively. Similar to other regulatory GTPases, they act as molecular switches cycling between an active GTP-bound state and an inactive GDP-bound state. This activity is controlled by (*a*) guanine nucleotide exchange factors (GEFs) that catalyze exchange of GDP for GTP to activate the switch (Schmidt & Hall 2002); (*b*) GTPase-activating proteins (GAPs) that stimulate the intrinsic GTPase activity to inactivate the switch (Bernards 2003); and (*c*) guanine nucleotide dissociation inhibitors (GDIs), whose role appears to be to block spontaneous activation (Olofsson 1999) (**Figure 1**). Interestingly, the three Rnd proteins and RhoH lack any de-

tectable GTPase activity and so it is not clear that they should be regarded as molecular switches, at least in the conventional sense. Finally, Rho GTPases can be regulated through direct phosphorylation or ubiquitination (Lang et al. 1996, Wang et al. 2003), but the extent to which these covalent modifications play a role in normal physiology is unclear.

It is in the active GTP-bound state that Rho GTPases perform their regulatory function through a conformation-specific interaction with target (effector) proteins. Over 50 effectors have been identified so far for Rho, Rac, and Cdc42 that include serine/threonine kinases, tyrosine kinases, lipid kinases, lipases, oxidases, and scaffold proteins. For the handful of targets that have been examined structurally, it appears that they exist in a closed inactive conformation that is relieved through GTPase binding (Bishop & Hall 2000). However, it is possible that GTPases might also serve to recruit targets to specific locations or complexes.

BIOCHEMICAL FUNCTIONS

Actin Cytoskeleton

The activation of Rho, Rac, or Cdc42 leads to the assembly of contractile actin:myosin filaments, protrusive actin-rich lamellipodia, and protrusive actin-rich filopodia, respectively (Etienne-Manneville & Hall 2002). These highly specific effects on the actin cytoskeleton point to a series of well-defined signal transduction pathways controlled by each GTPase leading to both the formation (actin polymerization) and the organization (filament bundling) of actin filaments.

Actin polymerization. Actin polymerization in eukaryotic cells occurs through the coordinated activities of filament severing and capping proteins and the two major actin polymerization factors Arp2/3 and Formin.

Arp2/3. Although Rac and Cdc42 lead to morphologically distinct protrusions at the
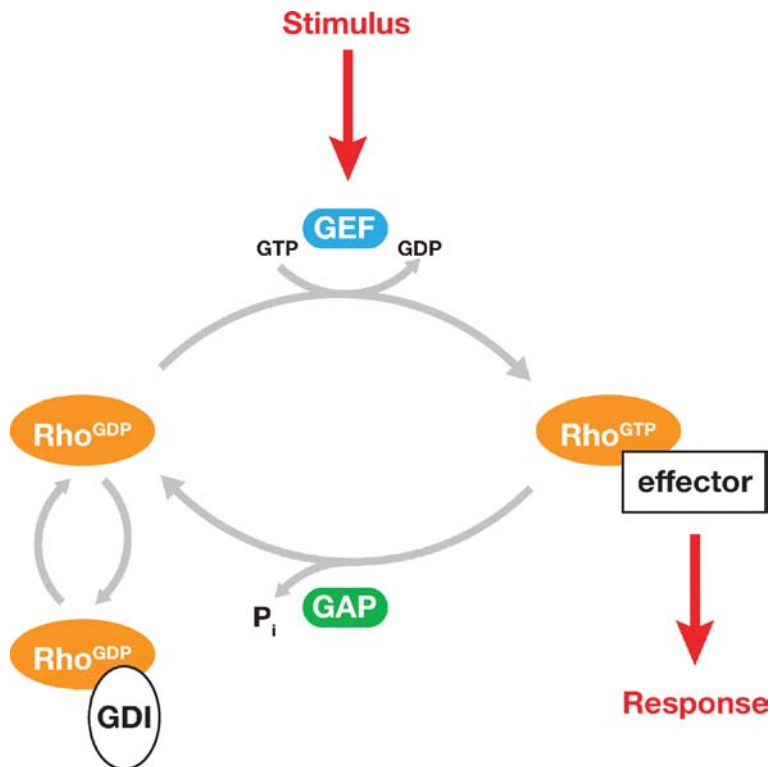
plasma membrane (i.e., lamellipodia and filopodia), they both initiate peripheral actin polymerization through the Arp2/3 complex. This heptameric, actin-nucleation machine is found in all eukaryotic cells; it associates with the sides and, perhaps, also the ends of existing actin filaments to initiate new branched filaments (Millard et al. 2004) (**Figure 2**). Both GTPases activate Arp2/3 indirectly through members of the Wiskott-Aldrich syndrome protein (WASP) family. In vitro, Cdc42.GTP binds directly to N-WASP, or the closely related, hemopoietic-specific WASP, to relieve an intra-molecular, auto-inhibitory interaction and expose a C-terminal Arp2/3 binding/activation site. However, recent work has shown that the situation may be more complicated, with the majority of cellular N-WASP bound to a protein, WIP or CR16, which suppresses activation by Cdc42 (Ho et al. 2001, Martinez-Quiles et al. 2001). This suggests that in vivo N-WASP may be *trans*-inhibited

rather than auto-inhibited, and indeed a new Cdc42 target, Toca-1 (transducer of Cdc42-dependent actin assembly), has been identified that is required for activation of the N-WASP/WIP complex (Ho et al. 2004). The significance of having two direct targets of Cdc42 (i.e., N-WASP and Toca-1) in the signal transduction pathway to Arp2/3 is unclear; perhaps it allows more finely regulated control.

Activation of Arp2/3 by Rac is mediated by WAVE family proteins (**Figure 2**), which although structurally related to N-WASP, do not interact directly with the GTPase. Progress in understanding this signal transduction pathway has also come from a biochemical approach, which has led to the isolation of a complex containing WAVE1, along with three other proteins, HSPC300, Nap125, and PIR121 (Eden et al. 2002). Nap125 and PIR121 are both direct Rac targets, and it has been proposed that Rac
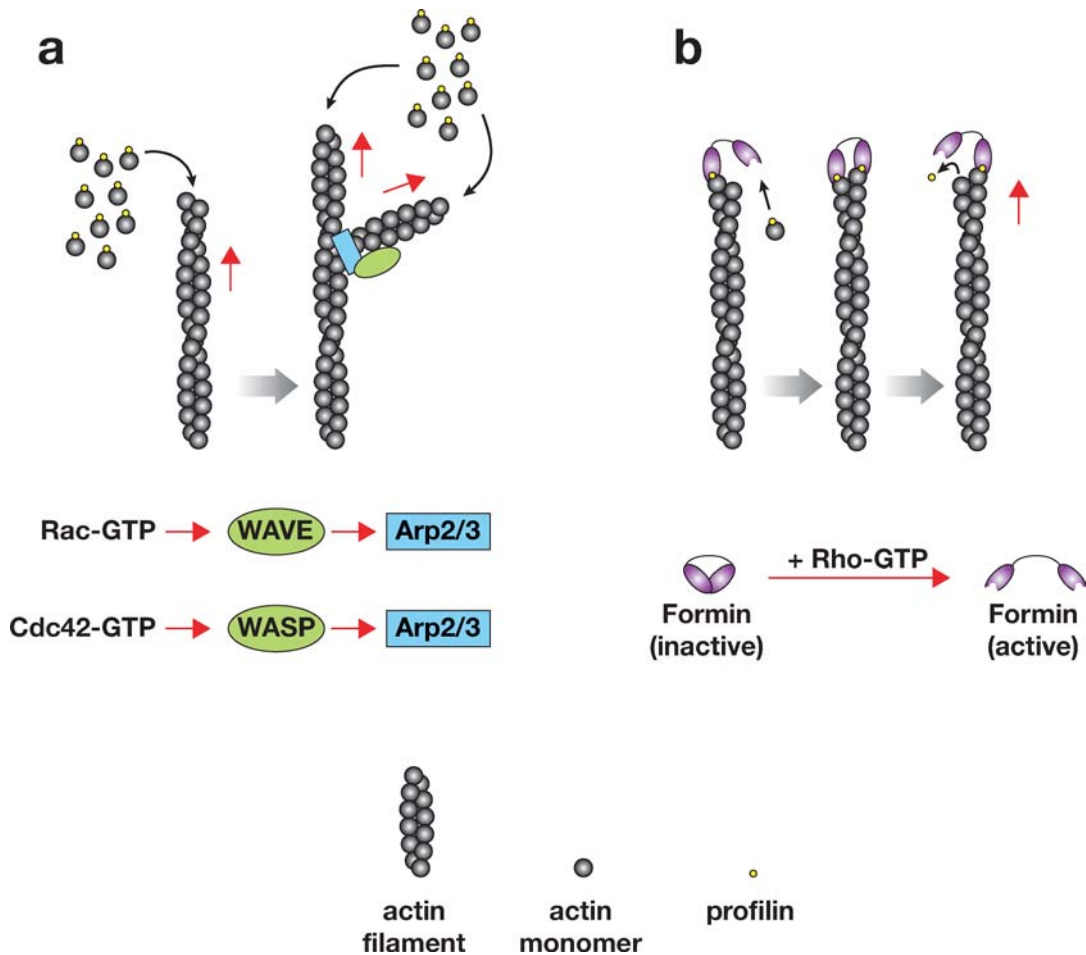
Figure 2

Rho GTPases regulate the two major modes of actin polymerization. (*a*) Rac and Cdc42 activate Arp2/3 via WAVE (a WASP family protein) and WASP, respectively, to initiate a branched filament network. (*b*) Rho activates formins to promote linear elongation of filaments at barbed ends.

promotes the disassembly of this inactive complex, allowing WAVE to interact directly with Arp2/3. A second group has isolated a similar complex, containing WAVE2, but this has an additional component, Abi1, and is active in actin polymerization assays (Innocenti et al. 2004). This group argues that the role of Rac is to localize this complex to the cell periphery to promote actin nucleation. Further analysis is required to resolve the issue.

Formins. The other major mechanism for inducing actin polymerization in eukaryotic

cells is through the formin family of proteins. In particular, Rho stimulates actin polymerization in mammalian cells through the diaphanous-related formin (DRF), mDia1 (and possibly mDia2), and in *S. cerevisiae* through Bnr1 and Bni1, the only two formins in this organism. mDia1 is a direct target of Rho. GTP and binding of the GTPase relieves an auto-inhibitory interaction, exposing an FH2 domain that then binds to the barbed end of an actin filament (Zigmond 2004). mDia1 also contains an essential FH1 domain, which interacts with a profilin/actin

complex and delivers it to the filament end. The remarkable thing here is that once mDia1 has added an actin monomer, it remains bound to the barbed end ready to add another actin monomer. This processive mechanism of filament elongation has been described as a leaky cap and is schematically depicted in **Figure 2**. Exactly how monomer assembly occurs at the barbed end while the formin is still bound is unclear; most other barbed end–binding proteins inhibit filament elongation. Given the complexity found with Arp2/3 activation, it would not be surprising to find that mDia is part of a larger complex and subject to further regulation.

Cofilin. ADF/cofilin severs actin filaments, leading to an increase in uncapped barbed ends that serve as sites for actin polymerization and filament elongation (Ghosh et al. 2004). Cofilin also participates in filament disassembly by promoting actin monomer dissociation from the pointed end, and both activities seem to be important for productive membrane protrusions (Dawe et al. 2003, Desmarais et al. 2005, Pollard & Borisy 2003). Cofilin is tightly regulated, and its activity is affected by phosphorylation, PIP_2 binding, changes in intracellular pH, and protein-protein interactions. Phosphorylation of cofilin leads to inactivation and occurs primarily through LIM kinases (LIMK), which in turn are activated by the PAK family of Rac/Cdc42-dependent kinases. How to reconcile the requirement for both active Rac and active (i.e., non-phosphorylated) cofilin at sites of membrane protrusion is not yet clear, but may involve spatially distinct compartments (Dawe et al. 2003, Svitkina & Borisy 1999). LIMK-dependent phosphorylation of cofilin can also be induced by Rho acting through its target Rho kinase (ROCK), and this may be an important event in the stabilization of actin:myosin filaments (Ohashi et al. 2000).

Actin filament organization. In addition to elongation, the discrete changes to the actin cytoskeleton induced by Rho, Rac, or Cdc42 require the correct spatial organization of filaments. This has been best characterized for Rho-induced assembly of contractile actin:myosin filaments, which is mediated by ROCK. Although this serine/threonine kinase has many substrates, the key event appears to be phosphorylation-induced inactivation of myosin light chain (MLC) phosphatase (Riento & Ridley 2003). This in turn leads to increased phosphorylation of MLC, which promotes the actin filament cross-linking activity of myosin II.

Less is known about how Rac and Cdc42 organize actin filaments into branched and unbranched filaments, respectively. Recent studies suggest that the formation of unbranched bundles of actin filaments found in filopodia originate from a branched network initiated by Cdc42 activation of Arp2/3 (Svitkina et al. 2003). Remodeling then occurs through a combination of (*a*) inhibiting barbed end capping to allow filament elongation and (*b*) promoting filament cross-linking through actin-bundling proteins such as fascin (Vignjevic et al. 2003).

Microtubule Cytoskeleton

Similar to actin filaments, microtubules have an intrinsic polarity, with a minus end (usually, but not always, anchored at the centrosome) and a dynamic plus end (usually at the cell periphery). The change from growth to shrinking (catastrophe) and from shrinking to growth (rescue) at the plus end is referred to as dynamic instability. In addition, the intracellular organization of microtubules makes a major contribution to cell polarity and to the distribution of intracellular organelles, such as the Golgi and mitotic spindle.

Microtubule dynamics. Microtubule plus end–binding proteins profoundly influence microtubule dynamics, and Rho GTPases can regulate this in different ways. The Op18/stathmin family, for example, interacts

both with microtubule plus ends to promote catastrophic disassembly and with tubulin dimers to inhibit polymerization (Cassimeris 2002). Op18/stathmin can be phosphorylated at four key residues, any one of which leads to its inactivation, thereby resulting in net elongation of microtubule ends. Phosphorylation at Ser16 is mediated by Cdc42/Rac-dependent activation of PAK, which occurs in response to a number of extracellular stimuli (Daub et al. 2001).

The effect of Rho on microtubule dynamics is likely to be context dependent. In neurons, collapsin response mediator protein-2 (CRMP-2) binds tubulin heterodimers and promotes microtubule assembly, perhaps by delivering dimers to the plus ends of growing microtubules (Y. Fukata et al. 2002). CRMP-2 is phosphorylated and inactivated by ROCK (at Thr555), which correlates well with growth cone collapse induced by LPA, although not by semaphorin 3A (Arimura et al. 2000). In migrating fibroblasts, on the other hand, Rho promotes the formation of stabilized microtubules, as visualized by an increase in detyrosinated tubulin. It is not clear how stabilization occurs; it appears to be mediated by mDia but does not involve changes to the actin cytoskeleton (Palazzo et al. 2001).

Microtubule plus end capture. Microtubules play a major role in defining cell shape and polarity through the specific interaction of their plus ends with proteins at the cell cortex. This plus end capture of microtubules has been attributed to a number of plus end–binding proteins, whose activities are influenced by Rho GTPases. CLIP-170, for example, can simultaneously bind to microtubules and to the scaffold protein IQGAP, a Rac/Cdc42 effector that is enriched at the leading edge of migrating cells. Expression of constitutively active Rac or Cdc42 enhances the ability of CLIP-170 to bind to IQGAP, thereby promoting plus end capture (M. Fukata et al. 2002). IQGAP was originally identified as a Rac/Cdc42 effector that can influence the actin cytoskele-

ton, and these observations therefore provide a potential biochemical link between the actin and microtubule cytoskeletons (Bashour et al. 1997).

Another plus end–binding protein, EB1, interacts with the adenomatous polyposis coli (APC) tumor suppressor protein; this not only stabilizes microtubules but also can facilitate interactions with proteins at the cell cortex. Two distinct pathways have been reported to regulate the APC/EB1 interaction and thereby promote microtubule capture. In fibroblasts, both EB1 and APC bind to mDia, suggesting a role for Rho in microtubule capture (Wen et al. 2004). In migrating astrocytes, the interaction of APC with EB1 at microtubule plus ends is regulated by Cdc42, acting through the Par6/PKCζ effector complex (Etienne-Manneville & Hall 2003). Interestingly, APC was recently found to bind to IQGAP, and both proteins are required for the proper maintenance of CLIP-170 at the leading edge of migrating cells (Watanabe et al. 2004).

Gene Expression

In addition to their cytoskeletal effects, Rho GTPases regulate several signal transduction pathways that lead to alterations in gene expression.

SRF—actin sensor. The serum response element (SRE) is found in many promoters, including those of genes encoding components of the cytoskeleton, most notably actin. Two transcription factors act at the SRE: (*a*) the ternary complex factor (TCF) regulated by the Ras/MAP kinase pathway and (*b*) the serum response factor (SRF) regulated by Rho. Recent work has established that SRF requires a co-activator, MAL, which translocates from the cytoplasm to the nucleus in response to Rho activation (Miralles et al. 2003). Furthermore, Rho-mediated changes to the actin cytoskeleton promote this translocation, although

precisely how is not clear. MAL binds to monomeric G-actin and actin polymerization results in MAL nuclear translocation, suggesting that MAL is sensitive to a decrease in cellular G-actin levels. However, it must be more complicated than this because nuclear translocation occurs even when the total levels of G-actin remain unchanged, and Rac and Cdc42, which are also strong inducers of actin polymerization, activate SRF poorly compared with activation by Rho.

Actin-independent pathways. Rho, Rac, and Cdc42 also affect gene transcription through signal transduction pathways not involving the actin cytoskeleton. All three GTPases are capable of activating the JNK and p38 MAP kinase pathway, although this is dependent on cell context, and there are many examples where they seem not to be involved (Coso et al. 1995, Minden et al. 1995, Puls et al. 1999). At least four MAP kinase kinase kinases (MAPKKKs) are direct targets of Rho GTPases: MLK2, MLK3, and MEKK4 interact with Rac/Cdc42, whereas MEKK1 interacts with Rho and with Rac/Cdc42, although through different sites (Burbelo et al. 1995, Gallagher et al. 2004, Teramoto et al. 1996).

Scaffold proteins play a major role in controlling the activation and specificity of MAP kinase pathways in vivo and, interestingly, at least three Rho GTPase targets, POSH, CNK, and MEKK1, act as scaffold proteins (Morrison & Davis 2003). POSH interacts with MLK2/3 (MAPKKK), MKK4/7 (MAPKK), and JNK (MAPK) and is required

for Rac-dependent activation of JNK in neurons upon NGF withdrawal, whereas CNK interacts with MLK2/3 and MKK7 and is required for Rho-dependent JNK activation upon serum addition to HeLa cells (Jaffe et al. 2005, Xu et al. 2003).

Rho, Rac, and Cdc42 have been reported to activate NFκB in response to a variety of stimuli, particularly inflammatory cytokines (Perona et al. 1997). A potential complication in elucidating the mechanism of GTPase activation of NFκB is that Rac and Cdc42 stimulate the production of reactive oxygen species (ROS) (see below) and inflammatory cytokines, both of which are potent activators of NFκB (Joneson & Bar-Sagi 1998, Kheradmand et al. 1998, Tapon et al. 1998). Therefore, it is still not entirely clear whether NFκB activation by Rho GTPases is direct or indirect.

Regulation of Enzymatic Activities

A number of additional enzymatic activities (**Table 1**) are influenced by Rho GTPases. Many are involved in lipid metabolism and some have been implicated in GTPase-mediated changes to the actin cytoskeleton (Tolias et al. 2000, Wang et al. 2002). One of the first targets of Rac to be identified was p67phox, an essential structural component of the NADPH oxidase complex found in phagocytic cells (Diekmann et al. 1994). Since then, Rac has been reported to promote ROS production in many cells, and a new family of widely expressed oxidases (Nox family) may mediate this activity (Lambeth 2002, Takeya et al. 2003).

Table 1   Additional enzymatic activities regulated by Rho GTPases

Enzymatic activity	Effector protein	GTPase	References
Lipid metabolism	PI4P 5-kinase	Rho, Rac	(Weernink et al. 2004)
	PI-3-kinase	Rac, Cdc42	(Zheng et al. 1994)
	DAG kinase	Rho, Rac	(Houssa et al. 1999)
	PLD	Rho, Rac, Cdc42	(Hess et al. 1997)
	PLC	Rac, Cdc42	(Illenberger et al. 1998)
ROS generation	NADPH oxidases	Rac	(Takeya & Sumimoto 2003)

BIOLOGICAL FUNCTIONS

Cell Cycle

The eukaryotic cell cycle consists of a DNA replication phase (S) and a nuclear/cell division phase (M) separated by two gap phases (G_1 and G_2). Rho GTPases influence the activity of cyclin-dependent kinases during G_1 and the organization of the microtubule and actin cytoskeletons during M (**Figure 3)**.

G_1 progression. Inhibition of Rho, Rac, or Cdc42 blocks G_1 progression in a variety of mammalian cell types, but the mechanisms are cell-type dependent and have proven difficult to elucidate (Olson et al. 1995, Yamamoto et al. 1993). G_1 progression is controlled by two types of cyclin-dependent kinases (Cdks), Cdk4/Cdk6 and Cdk2, which are activated by binding to cyclin D and cyclin E, respectively, and inhibited by binding to the INK4A and
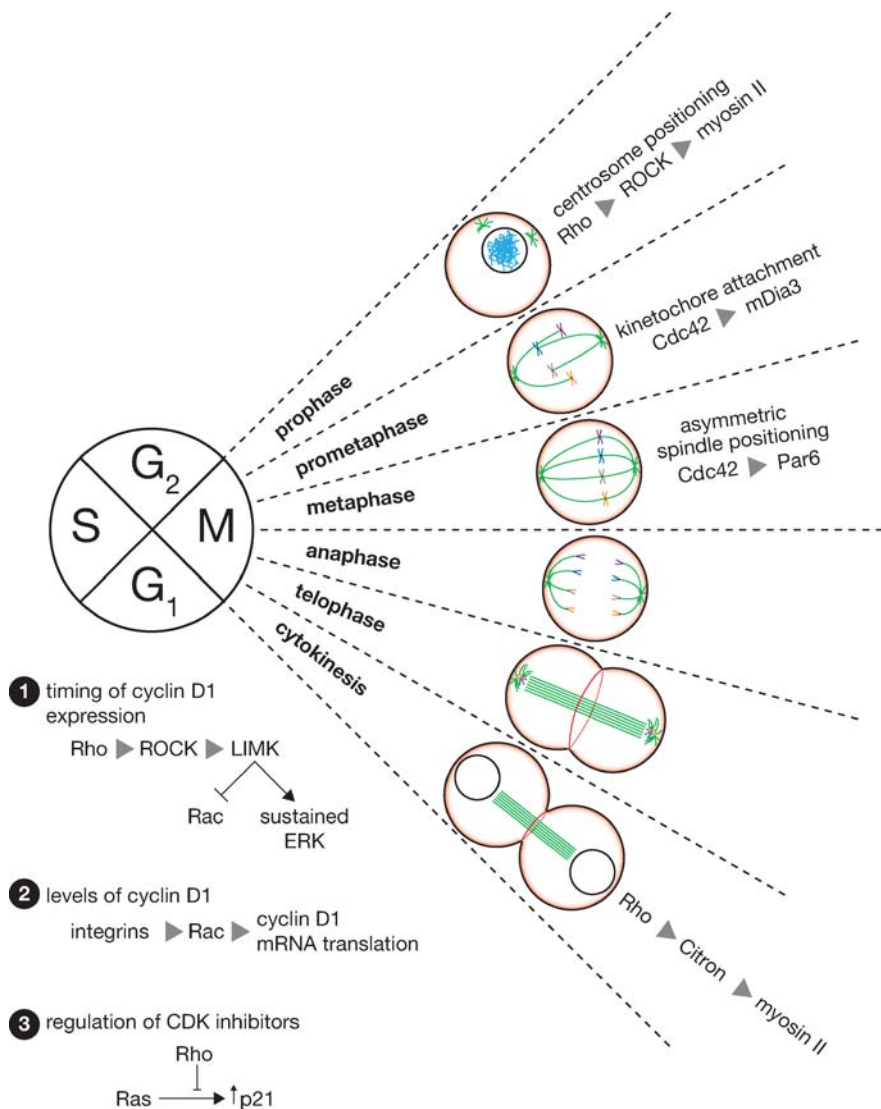

Figure 3

Rho GTPases and the cell cycle. Rho GTPases control multiple aspects of M phase and G_1 progression. The signaling pathways are shown for the relevant stages. Microtubules, green; actin, red; condensed chromosomes, blue.

Cip/Kip family of proteins. The key to G_1 progression lies in the cellular levels of cyclins and the Cdk inhibitors, and these are controlled by two major extrinsic factors: growth factors and extracellular matrix proteins.

The best understood route for affecting cyclin D levels is through growth factor–induced activation of transcription by the Ras/ERK pathway (Coleman et al. 2004). However, to achieve the correct cyclin D levels and at the right time (i.e., in mid G_1), ERK activation must be sustained. In normal cells this requires additional signal inputs from adhesion to the extracellular matrix. This phenomenon of adhesion- or anchorage-dependent proliferation is likely to account for many of the effects of Rho GTPases on G_1 progression.

There have been several reports that Rac and Cdc42 (but not Rho) stimulate cyclin D1 transcription when ectopically expressed in cells, and in one case at least, this was mediated by NFκB (Joyce et al. 1999, Westwick et al. 1997). More detailed analysis of endogenous Rho GTPases in NIH 3T3 fibroblasts has, however, painted a more complicated picture. In these cells, Rho is required for normal cyclin D expression, but Rho inhibition leads to premature cyclin D expression, which is Rac dependent (Welsh et al. 2001). The authors of this work argue that during the normal cell cycle, Rho acts to suppress Rac and to promote sustained activation of ERK, both of which are required for correct temporal control of cyclin D levels. Sustained ERK activity involves the Rho target kinase ROCK and its substrate LIMK, as well as the induction of stress fibers, whereas suppression of Rac is independent of actin and involves nuclear translocation of LIMK (Roovers & Assoian 2003, Roovers et al. 2003). How stress fibers influence ERK activity is unknown, and because cofilin is the only known substrate for LIMK, it is unclear how this actin-independent function of LIMK is mediated. A rather different story has emerged through analyzing G_1 progression in endothelial cells. Here too, matrix-dependent integrin activation is essential, but

this triggers a Rac pathway that controls cyclin D1 mRNA translation (Mettouchi et al. 2001). Finally, there has been one report that expression of cyclin E, which occurs in late G_1, can be stimulated by Cdc42 through its effector p70 S6 kinase (Chou et al. 2003). Unlike cyclin D1, the timing of cyclin E expression is independent of Rho kinase activity (Roovers & Assoian 2003).

Rho GTPases also regulate the levels of the Cdk2 inhibitors, p21[cip1] and p27[kip1]. The first indication of this came from studies showing that activated Ras is unable to stimulate G_1 progression in the absence of Rho. It appears that Ras promotes the accumulation of high levels of p21[cip1] and this is attenuated by Rho either through inhibition of p21[cip1] transcription or, possibly, through promotion of protein degradation (Olson et al. 1998, Weber et al. 1997). In fibroblasts and colon carcinoma cell lines, the effect is ROCK independent, but in other cell types, ROCK seems to be involved (Lai et al. 2002, Sahai et al. 2001). Rho regulation of p27[kip1] is posttranscriptional, although there are examples of it acting though protein degradation or mRNA translation (Hu et al. 1999, Vidal et al. 2002).

Many of the effects of Rho GTPases on G_1 progression are thought to reflect the crucial role of anchorage- or adhesion-dependent signals for cell proliferation. The loss of anchorage dependence is one of the hallmarks of cancer, which has sparked much interest in the possible contribution of deregulated Rho GTPase pathways to tumor progression (Jaffe & Hall 2002). In this respect, it is interesting to note that many members of the Rho GEF family can induce a transformed phenotype when expressed in immortalized cells.

Mitosis. The alignment of chromosomes during prophase and metaphase is driven primarily by microtubules emanating from the two centrosomes: Astral microtubules interact with the cell cortex, whereas spindle microtubules interact with the kinetochore. A major role of astral microtubules is to align the

spindle along an axis perpendicular to the future cell division plane, and it was recently shown that actin:myosin filaments, under the control of ROCK, are required at the cortex to allow positioning of the centrosomes (Rosenblatt et al. 2004). It is too early to say how myosin II promotes cortical movement of astral microtubules, but microtubules have been reported to influence Rho GTPases so localized inhibition of Rho (and therefore contractility) might be involved (Wittmann & Waterman-Storer 2001).

Another role of astral microtubules is to determine the position of the spindle. Although in cell culture this is symmetrical, producing two equal daughter cells after division, the asymmetric positioning of the spindle, giving rise to daughter cells of different size and specification, is crucially important both in development and in the adult (e.g., during stem cell division). Much of our understanding of this process has come from genetic screens in *C. elegans* and in particular, in the discovery of six Par proteins (Par1-6), along with atypical protein kinase C (aPKC), that are essential for asymmetric cell division in the zygote. These proteins are themselves asymmetrically distributed, with Par6/aPKC/Par3 at the anterior and Par1/Par2 at the posterior (the external cue here being the site of sperm entry), and they determine the positioning of the spindle so as to produce a large and a small daughter cell. The major interest here is that Par6 is a target for Cdc42, and this GTPase is also essential for asymmetric cell division (Gotta et al. 2001). Cdc42 induces a conformational change in Par6 and can activate aPKC, but precisely how this influences spindle positioning is still being elucidated (Ahringer 2003, Etienne-Manneville & Hall 2001, Peterson et al. 2004).

Spindle microtubules interact with chromosomes at the kinetochore, a complex of at least 50 proteins that includes the Cdc42-specific effector mDia3. Inhibition of Cdc42 or depletion of mDia3 causes a mitotic arrest in which many chromosomes are not properly attached to microtubules (Yasuda et al. 2004).

The GEF and GAP that control Cdc42 activity during this stage of mitosis have been identified as Ect2 and MgcRacGAP, respectively (Oceguera-Yanez et al. 2005). Interestingly, Cdc42-null ES cells proliferate normally, and although this could mean that spindle attachment here is GTPase independent, it more likely reflects some redundancy with the other close relatives of Cdc42, such as TCL or TC10 (Chen et al. 2000). The Cdc42-dependent attachment of microtubules to a kinetochore shows some similarities to the capture of microtubules at the cell cortex, and it will be interesting to see whether Cdc42 regulates the assembly of proteins at the tip of spindle microtubules as well as at the chromosomal attachment site (Narumiya et al. 2004). Finally, the mDia proteins are best known for their role in promoting actin polymerization (see above) leading to speculation that actin might play a role in spindle attachment.

Cytokinesis. Cell division is initiated at the end of mitosis through the assembly of a cleavage furrow and an associated contractile ring consisting of actin and myosin II filaments. Rho plays a crucial role in contractile ring function and localizes to the cleavage furrow along with at least three known effectors, ROCK, Citron kinase, and mDia (Glotzer 2001). Although the majority of actin filaments at the cleavage furrow originates from pre-existing filaments, it is likely that some de novo actin polymerization is required and perhaps this is the role of mDia (Kato et al. 2001). The respective roles of the two Rho-dependent kinases have been difficult to tease out. Myosin is activated by phosphorylation of its light chain (MLC) at Thr18 and Ser19, and this drives contraction of the actin filament ring (Komatsu et al. 2000, Matsumura et al. 1998). Citron kinase phosphorylates MLC directly at both sites, whereas ROCK affects these sites indirectly through phosphorylation and inhibition of MLC phosphatase (Yamashiro et al. 2003). Inhibition of Citron kinase, but not ROCK, blocks cytokinesis in HeLa cells; however, in other cell types

ROCK is also important, and the phenotype of the Citron kinase knockout mice, although severe, is not lethal (Di Cunto et al. 2000, Kosako et al. 2000, Madaule et al. 1998). The dynamic changes in contractile forces during the division process are likely to be complex and perhaps both kinases play distinct, but partially overlapping roles. In this respect it is interesting that when ectopically expressed, Citron kinase can replace ROCK in stress fiber assembly (Yamashiro et al. 2003). Finally, the GEF and GAP responsible for regulating Rho activity during cytokinesis are Ect2 and MgcRacGAP (Lee et al. 2004, Prokopenko et al. 1999, Tatsumoto et al. 1999). This raises some very interesting questions concerning regulation and specificity, since earlier in mitosis the same GEF and GAP apparently con-

trol Cdc42 activity to promote kinetochore attachment (Oceguera-Yanez et al. 2005).

Cell Morphogenesis

Cell morphology is intimately linked to function. In response to external cues, Rho GTPases contribute to morphogenesis by regulating both the actin and microtubule cytoskeletons and the core machinery involved in establishing polarity (**Figure 4**).

Cell-cell interactions. The assembly of molecularly distinct cell-cell adhesion complexes and the concomitant establishment of polarity drive the morphogenesis of many cell types. In epithelial cells, E-cadherin,
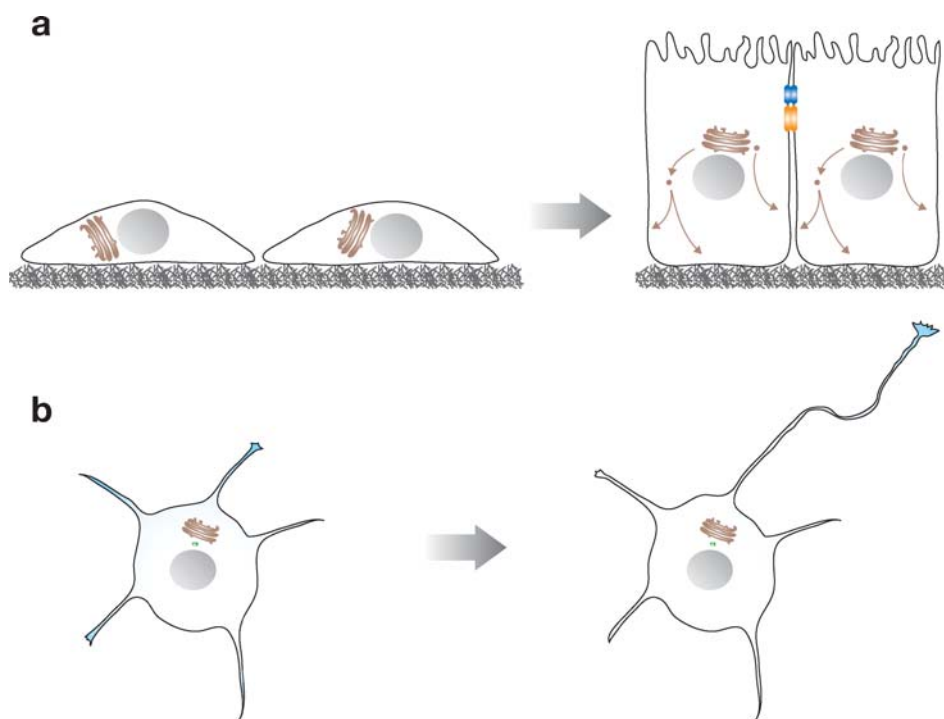

Figure 4

Rho GTPases and cell morphogenesis. (*a*) Epithelial morphogenesis involves cell-cell interactions leading to the formation of apical and basolateral domains, separated by tight junctions (*blue*) and adherens junctions (*orange*). Cell polarity is reinforced through the directed trafficking of vesicles from the Golgi (*brown*). (*b*) Neuronal morphogensis begins with the establishment of axonal and somato-dendritic compartments.

a member of a family of Ca^{2+}-dependent *trans*-membrane proteins, participates in homophilic interactions to produce stable adherens junctions through the subsequent recruitment of intracellular proteins (such as α- and β-catenin) and the actin cytoskeleton. Rho, Rac, and Cdc42 have each been implicated in adherens junction assembly.

The inhibition of Rho or Rac in keratinocytes prevents the formation of adherens junctions, whereas Tiam-1, a GEF for Rac, is essential for the formation and maintenance of junctions in Madin-Darby canine kidney (MDCK) cells plated on fibronectin (Braga et al. 1997, Malliri et al. 2004). Cadherin ligation leads to the recruitment of activated Rac to adhesion sites, where its major role is to stabilize the junction through the assembly of actin filaments (Hordijk et al. 1997, Takaishi et al. 1997). The Rac target IQGAP has been implicated in actin assembly at junctions; however, it has been suggested that IQGAP may also sequester β-catenin and that Rac and Cdc42 can disrupt this complex allowing β-catenin to participate in junction assembly (Kuroda et al. 1999, Noritake et al. 2004). An additional complication to the story is that hyperactivation of Rac in keratinocytes leads to junction disassembly, and activation of Rac in MDCK cells plated on collagen promotes migration rather than cell-cell adhesion (Braga et al. 2000, Sander et al. 1998). Because Rac is involved in two seemingly opposing activities, namely cell-cell junction assembly and cell migration, it is likely that its effects will be greatly influenced by environmental factors and cell type. A major role for Rho at junctions is probably through its effector ROCK and F-actin assembly, although it too has been shown to interact directly with junctional proteins (α-catenin and p120ctn), at least in *Drosophila* (Magie et al. 2002, Vaezi et al. 2002).

Numerous reports indicate that adherens junction assembly is preceded by the localized induction of filopodia and/or lamellipodia and that these protrusive structures drive intimate membrane contact between adjacent cells (Ehrlich et al. 2002, Jacinto et al.

2000, Vasioukhin et al. 2000). The formation of localized protrusions may be initiated by early cadherin interactions that activate Rac, and perhaps Cdc42, which could then generate a positive feedback loop (Noritake et al. 2004). However, an alternative possibility has emerged from work on a relatively new family of cell-cell adhesion proteins, the nectins. These Ca^{2+}-independent, immunoglobulin-like *trans*-membrane proteins form homo- and heteromolecular interactions, which suggests that this is required for subsequent cadherin-based junctional assembly (Irie et al. 2004, Kawakatsu et al. 2002). Nectin-nectin interactions activate both Cdc42 and Rac, and this could provide the localized protrusive activity that facilitates subsequent cadherin-based adhesion (Fukuhara et al. 2004).

Cell polarity. The morphogenesis of epithelial cells requires the establishment of cell polarity to form apical and basolateral domains, which involves the assembly of tight junctions formed by homophilic interactions involving another family of integral membrane proteins, the claudins. Although the details of how tight junctions are assembled apically to adherens junctions are far from well understood, genetic analyses in flies and worms have identified some key players in this process. Specifically, three protein complexes have been implicated in epithelial morphogenesis: Par6/aPKC/Par3, Dlg/Lgl/scribble, and Crumbs/PALS1/PATJ (Gibson & Perrimon 2003).

As described above, Par6 is a direct target of Cdc42 and is required for asymmetric cell division. Although morphogenesis is a very different biological process, it also involves the establishment of asymmetry through the specification of distinct domains around the cell periphery. Work with cultured mammalian epithelial cells has shown that the Par6/aPKC/Par3 complex localizes apically with tight junctions, whereas Par1 localizes laterally (Izumi et al. 1998). Par3 binds directly to JAM, a *trans*-membrane adhesion protein that interacts directly with a core,

tight junctional component, ZO-1. When overexpressed, Par3 promotes the formation of tight junctions (Hirose et al. 2002, Itoh et al. 2001). Furthermore, the Cdc42-dependent activation of aPKC is required for junction assembly (Suzuki et al. 2001, Yamanaka et al. 2001). One of the substrates of aPKC is Lgl, and recently Cdc42 and aPKC have been shown to induce localization of Dlg to the front of migrating cells (Plant et al. 2003, Yamanaka et al. 2003, S. Etienne-Manneville & A. Hall, unpublished results). These observations suggest that in some situations Cdc42 may signal via the Par6/aPKC to regulate the Dlg/Lgl/scribble complex. Other observations point to a more complex relationship between the Par complex and tight junction assembly. For example, Par3 inhibits aPKC activity in vitro, and in one report Par6 negatively regulates tight junction formation (Gao et al. 2002, Lin et al. 2000).

Epithelial morphogenesis in vivo requires additional signals to establish polarity within the context of a tissue (Zegers et al. 2003). Extracellular matrix provides a likely signal for defining the appropriate orientation of the apical surface and in vitro three-dimensional morphogenesis assays have uncovered a potential role for Rac in this process (Yu et al. 2005). Finally, polarization may also take place across tissues along the proximal-distal axis referred to as planar polarity (Fanto & McNeill 2004). Work in the *Drosophila* eye and wing have shed most light on this process, and it appears that Rac and Rho, but not Cdc42, acting downstream of the Dishevelled protein and the family of Frizzled receptors, play a key role. The effects of Rac and Rho are complex, but it interesting to note that they likely involve both the actin cytoskeleton and JNK-dependent gene transcription.

Although the initiating signals are different, neuronal morphogenesis also involves Cdc42 and the Par proteins. When hippocampal neurons are plated in culture, they spontaneously polarize, in the absence of cell-cell contacts, to form a single axon and a somato-dendritic compartment (extracellular cues presumably control this response in vivo) (**Figure 4**). Cdc42 and the Par3/Par6/aPKC complex are specifically enriched in the developing axon and disruption of their activity results in polarity defects, leading to zero or multiple axons (Schwamborn & Puschel 2004, Shi et al. 2003). Localized inhibition of GSK-3 is required in the presumptive axon, and this promotes the polarized localization of Par3 and two microtubule plus end–binding proteins, APC and CRMP-2 (Jiang et al. 2005, Shi et al. 2004, Yoshimura et al. 2005). It is interesting to compare this effect with polarity establishment in migrating cells (see below), where the localized accumulation of APC requires Par/aPKC-dependent inhibition of GSK-3 (Etienne-Manneville & Hall 2003).

Concomitant with the establishment of polarity, the morphogenetic program in neurons and epithelia is reinforced by the polarized trafficking of vesicles. In MDCK cells, for example, Cdc42 is required for the sorting of proteins to the basolateral surface, although not to the apical surface (Kroschewski et al. 1999). The small GTPase Ral and its effector Sec5, a component of the exocyst complex, have been implicated in Cdc42-induced filopodia formation in fibroblasts (Sugihara et al. 2002), raising the possibility that Cdc42 regulates the exocyst in mammalian cells, as it does in yeast, to influence basolateral trafficking (Sugihara et al. 2002, Zhang et al. 2001). Finally, Rho GTPases may regulate vesicular trafficking by regulating the microtubule cytoskeleton, which is dramatically reorganized during both epithelial and neuronal morphogenesis (Musch 2004).

Cell Migration

Actin polymerization and filament elongation at the front, coupled to actin:myosin filament contraction at the rear, are thought to provide the major driving forces for migration in animal cells. Polarization of these two activities can occur spontaneously and is often seen with single cells in culture, but this is usually short-lived and leads to random migration.
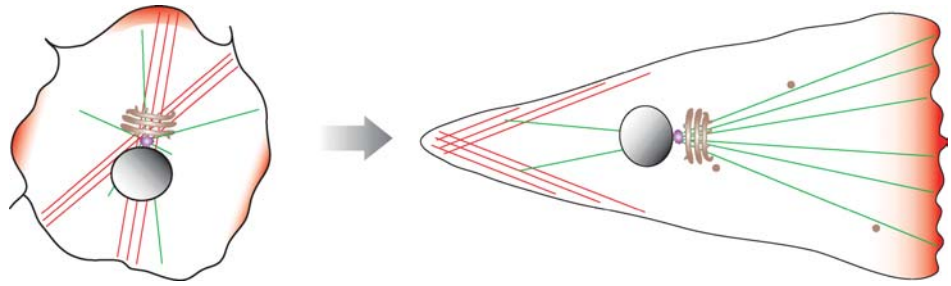
Figure 5

Rho GTPases and cell migration. Cell migration requires actin-dependent protrusions at the front (*red*) and contractile actin:myosin filaments (*red*) at the rear. In addition, microtubules (*green*) originating from the centrosome (*purple*) are preferentially stabilized in the direction of migration allowing targeted vesicle trafficking from the Golgi (*brown*) to the leading edge.

In vivo, cell migration is directed and extracellular cues polarize the actin cytoskeleton accordingly. Persistent and efficient directed migration requires additional cellular changes involving polarization of the microtubule cytoskeleton and the secretory pathway (Ridley et al. 2003) (**Figure 5**).

Movement. Rac, acting through WAVE and Arp2/3, is required to promote actin polymerization at the front of migrating cells, and this pushes forward the leading edge membrane. GTP-bound active Rac accumulates at the front of migrating cells, and in chemotaxing neutrophils this is sustained through a positive feedback loop with PI 3-kinase and its lipid product PIP_3 (Gardiner et al. 2002, Itoh et al. 2002, Kraynov et al. 2000). Work in *Dictyostelium* and neutrophils has shown that this polarized accumulation of PIP_3 is reinforced through localization of the lipid phosphatase PTEN to the sides and rear of the migrating cell (Li et al. 2003, Merlot & Firtel 2003).

This scenario of Rac activation at the front guiding movement is almost certainly an oversimplification. For example, fluorescence resonance energy transfer (FRET) -based assays have revealed the presence of Rac.GTP at the rear as well as the front of migrating neutrophils (Gardiner et al. 2002). Because protrusions are not found at the rear, it must be assumed that Rac is playing a different role in the two locations. One clear example where

Rac is known to make a contribution to cell migration other than through actin is during *Drosophila* dorsal closure. Here, Rac is required in leading edge cells not only for actin polymerization but also for JNK MAP kinase activation (Ricos et al. 1999). JNK signaling promotes transcription of the TGFβ−family member, Dpp, which when secreted acts on cells behind the leading edge to coordinate movement of the whole epithelial sheet (Glise & Noselli 1997, Hou et al. 1997). JNK may make other contributions to cell migration; it can, for example, phosphorylate paxillin, which leads to focal adhesion turnover, a prerequisite at the rear of cells for efficient movement (Huang et al. 2003).

Rho acts at the rear of the cell to generate contractile forces through ROCK-mediated MLC phosphorylation, which move the cell body forward (Riento & Ridley 2003). In addition, ROCK may inhibit inappropriate lateral protrusions, perhaps by restricting the formation of new integrin adhesion complexes (Worthylake & Burridge 2003). However, again things may not be so simple, and in some situations inhibition of ROCK stimulates cell migration (Nobes & Hall 1999). Furthermore, an E3 ubiquitin ligase, Smurf1, which ubiquitinates Rho, has been localized at the front of migrating cells (Wang et al. 2003). The significance of this is not clear—it would seem more economical not to activate Rho there in the first place, but perhaps it has

a role to play at the front and there is more to Rho ubiquitination than simply degradation.

More recently, cell migration studies using tumor cells in three-dimensional matrices have revealed striking differences from migration on two-dimensional tissue culture plates, which could have important implications in metastasis. Some tumor cells generate leading edge protrusive structures as might be expected, but they do not seem to require Rho or ROCK (Sahai & Marshall 2003). Presumably an alternative mechanism exists to promote actin:myosin contraction in the rear. However, another class of tumor cell moves with a rounded, blebbing morphology and here Rho and ROCK are essential. Rac is required for both types of migration (Sahai & Marshall 2003).

Directional sensing. The mechanisms by which external cues direct Rac and the activation of Arp2/3 to the leading edge and thereby determine the direction of migration (directional sensing) are less well characterized, but in some cases, at least, this involves Cdc42. The first indication that Cdc42 links extracellular cues to intracellular polarity came through studies on the pheromone-mating response in yeast, and this was later confirmed by work in animal cells using either single cells moving in a chemotactic gradient or sheets of cells moving in an in vitro scratch or wound assay (Allen et al. 1998, Nobes & Hall 1999, Simon et al. 1995). For example, Cdc42 is essential for the directional migration of a macrophage cell in a gradient of the chemoattractant M-CSF1, and when this GTPase is inhibited, cells still move, but do so randomly (Allen et al. 1998). Inhibition of Rac in the same cells blocks migration totally. Similarly, Cdc42 is essential for restricting Rac-dependent actin polymerization to the front of fibroblasts induced to migrate by scratching a monolayer (Cau & Hall 2005). It appears, therefore, that Cdc42 is locally activated by external cues (chemoattractant or loss of cell-cell contact) and then activates a pathway that determines the spatial localization of active Rac. Recent work in neutrophils suggests that this involves the localization of α-PIX, a Rac-specific GEF, through an unexpected pathway in which the Cdc42 target, PAK, acts as a scaffold rather than a kinase (Li et al. 2003).

Directed migration also involves polarization of the microtubule cytoskeleton, readily visualized in most, although not all, cell types through reorientation of the centrosome to the side of the nucleus facing the direction of migration. Cdc42 also regulates this aspect of cell polarity through a mechanism that involves some interesting parallels with polarity establishment during morphogenesis and asymmetric cell division. In particular, active Cdc42 at the front of the migrating cell leads to localized activation of the atypical PKC in the Par6/aPKC complex (Etienne-Manneville & Hall 2001). This has at least two consequences important for establishing microtubule polarity: (*a*) inactivation of the serine/threonine kinase GSK3 to promote association of APC with microtubule plus ends preferentially at the leading edge (Etienne-Manneville & Hall 2003) and (*b*) association of Dlg with the leading edge cortex (S. Etienne-Manneville & A. Hall, unpublished results). The interaction between microtubule-bound APC and cortex-bound Dlg is required for microtubule polarization (S. Etienne-Manneville & A. Hall, unpublished results).

CONCLUSIONS

It is impossible to present the whole range of biochemical pathways and biological processes that are influenced by Rho GTPases in a single review. Instead, we have tried to focus on areas that are better characterized and that provide more general insights into cellular functions. It has been disappointing not to be able to do justice to neuronal morphogenesis or to cover host-pathogen interactions and developmental processes, but perhaps this is better left to experts in those areas; the interested reader will certainly have no problem in finding suitable reviews.

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LITERATURE CITED

Ahringer J. 2003. Control of cell polarity and mitotic spindle positioning in animal cells. *Curr. Opin. Cell Biol.* 15:73–81

Allen WE, Zicha D, Ridley AJ, Jones GE. 1998. A role for Cdc42 in macrophage chemotaxis. *J. Cell Biol.* 141:1147–57

Arimura N, Inagaki N, Chihara K, Menager C, Nakamura N, et al. 2000. Phosphorylation of collapsin response mediator protein-2 by Rho-kinase. Evidence for two separate signaling pathways for growth cone collapse. *J. Biol. Chem.* 275:23973–80

Aspenstrom P, Fransson A, Saras J. 2004. Rho GTPases have diverse effects on the organization of the actin filament system. *Biochem. J.* 377:327–37

Bashour AM, Fullerton AT, Hart MJ, Bloom GS. 1997. IQGAP1, a Rac- and Cdc42-binding protein, directly binds and cross-links microfilaments. *J. Cell Biol.* 137:1555–66

Bernards A. 2003. GAPs galore! A survey of putative Ras superfamily GTPase activating proteins in man and *Drosophila*. *Biochim. Biophys. Acta* 1603:47–82

Bishop AL, Hall A. 2000. Rho GTPases and their effector proteins. *Biochem. J.* 348(Pt 2):241–55

Braga VM, Betson M, Li X, Lamarche-Vane N. 2000. Activation of the small GTPase Rac is sufficient to disrupt cadherin-dependent cell-cell adhesion in normal human keratinocytes. *Mol. Biol. Cell* 11:3703–21

Braga VM, Machesky LM, Hall A, Hotchin NA. 1997. The small GTPases Rho and Rac are required for the establishment of cadherin-dependent cell-cell contacts. *J. Cell Biol.* 137:1421–31

Burbelo PD, Drechsel D, Hall A. 1995. A conserved binding motif defines numerous candidate target proteins for both Cdc42 and Rac GTPases. *J. Biol. Chem.* 270:29071–74

Cau J, Hall A. 2005. Cdc42 controls the polarity of the actin and microtubule cytoskeletons through two distinct signal transduction pathways. *J. Cell Sci.* 118(Pt. 12):2579–87

Cassimeris L. 2002. The oncoprotein 18/stathmin family of microtubule destabilizers. *Curr. Opin. Cell Biol.* 14:18–24

Chen F, Ma L, Parrini MC, Mao X, Lopez M, et al. 2000. Cdc42 is required for PIP_2-induced actin polymerization and early development but not for cell viability. *Curr. Biol.* 10:758–65

Chou MM, Masuda-Robens JM, Gupta ML. 2003. Cdc42 promotes G1 progression through p70 S6 kinase-mediated induction of cyclin E expression. *J. Biol. Chem.* 278:35241–47

Coleman ML, Marshall CJ, Olson MF. 2004. RAS and RHO GTPases in G1-phase cell-cycle regulation. *Nat. Rev. Mol. Cell Biol.* 5:355–66

Coso OA, Chiariello M, Yu JC, Teramoto H, Crespo P, et al. 1995. The small GTP-binding proteins Rac1 and Cdc42 regulate the activity of the JNK/SAPK signaling pathway. *Cell* 81:1137–46

Daub H, Gevaert K, Vandekerckhove J, Sobel A, Hall A. 2001. Rac/Cdc42 and p65PAK regulate the microtubule-destabilizing protein stathmin through phosphorylation at serine 16. *J. Biol. Chem.* 276:1677–80

Dawe HR, Minamide LS, Bamburg JR, Cramer LP. 2003. ADF/cofilin controls cell polarity during fibroblast migration. *Curr. Biol.* 13:252–57

Desmarais V, Ghosh M, Eddy R, Condeelis J. 2005. Cofilin takes the lead. *J. Cell Sci.* 118:19–26

Di Cunto F, Imarisio S, Hirsch E, Broccoli V, Bulfone A, et al. 2000. Defective neurogenesis in citron kinase knockout mice by altered cytokinesis and massive apoptosis. *Neuron* 28:115–27

Diekmann D, Abo A, Johnston C, Segal AW, Hall A. 1994. Interaction of Rac with p67phox and regulation of phagocytic NADPH oxidase activity. *Science* 265:531–33

Eden S, Rohatgi R, Podtelejnikov AV, Mann M, Kirschner MW. 2002. Mechanism of regulation of WAVE1-induced actin nucleation by Rac1 and Nck. *Nature* 418:790–93

Ehrlich JS, Hansen MD, Nelson WJ. 2002. Spatio-temporal regulation of Rac1 localization and lamellipodia dynamics during epithelial cell-cell adhesion. *Dev. Cell* 3:259–70

Etienne-Manneville S, Hall A. 2001. Integrin-mediated activation of Cdc42 controls cell polarity in migrating astrocytes through PKCzeta. *Cell* 106:489–98

Etienne-Manneville S, Hall A. 2002. Rho GTPases in cell biology. *Nature* 420:629–35

Etienne-Manneville S, Hall A. 2003. Cdc42 regulates GSK-3beta and adenomatous polyposis coli to control cell polarity. *Nature* 421:753–56

Fanto M, McNeill H. 2004. Planar polarity from flies to vertebrates. *J. Cell Sci.* 117:527–33

Fukata M, Watanabe T, Noritake J, Nakagawa M, Yamaga M, et al. 2002. Rac1 and Cdc42 capture microtubules through IQGAP1 and CLIP-170. *Cell* 109:873–85

Fukata Y, Itoh TJ, Kimura T, Menager C, Nishimura T, et al. 2002. CRMP-2 binds to tubulin heterodimers to promote microtubule assembly. *Nat. Cell Biol.* 4:583–91

Fukuhara T, Shimizu K, Kawakatsu T, Fukuyama T, Minami Y, et al. 2004. Activation of Cdc42 by *trans* interactions of the cell adhesion molecules nectins through c-Src and Cdc42-GEF FRG. *J. Cell Biol.* 166:393–405

Gallagher ED, Gutowski S, Sternweis PC, Cobb MH. 2004. RhoA binds to the amino terminus of MEKK1 and regulates its kinase activity. *J. Biol. Chem.* 279:1872–77

Gao L, Joberty G, Macara IG. 2002. Assembly of epithelial tight junctions is negatively regulated by Par6. *Curr. Biol.* 12:221–25

Gardiner EM, Pestonjamasp KN, Bohl BP, Chamberlain C, Hahn KM, Bokoch GM. 2002. Spatial and temporal analysis of Rac activation during live neutrophil chemotaxis. *Curr. Biol.* 12:2029–34

Ghosh M, Song X, Mouneimne G, Sidani M, Lawrence DS, Condeelis JS. 2004. Cofilin promotes actin polymerization and defines the direction of cell motility. *Science* 304:743–46

Gibson MC, Perrimon N. 2003. Apicobasal polarization: epithelial form and function. *Curr. Opin. Cell Biol.* 15:747–52

Glise B, Noselli S. 1997. Coupling of Jun amino-terminal kinase and Decapentaplegic signaling pathways in *Drosophila* morphogenesis. *Genes Dev.* 11:1738–47

Glotzer M. 2001. Animal cell cytokinesis. *Annu. Rev. Cell Dev. Biol.* 17:351–86

Gotta M, Abraham MC, Ahringer J. 2001. CDC-42 controls early cell polarity and spindle orientation in *C. elegans*. *Curr. Biol.* 11:482–88

Hess JA, Ross AH, Qiu RG, Symons M, Exton JH. 1997. Role of Rho family proteins in phospholipase D activation by growth factors. *J. Biol. Chem.* 272:1615–20

Hirose T, Izumi Y, Nagashima Y, Tamai-Nagai Y, Kurihara H, et al. 2002. Involvement of ASIP/PAR-3 in the promotion of epithelial tight junction formation. *J. Cell Sci.* 115:2485–95

Ho HY, Rohatgi R, Lebensohn AM, Le M, Li J, et al. 2004. Toca-1 mediates Cdc42-dependent actin nucleation by activating the N-WASP-WIP complex. *Cell* 118:203–16

Ho HY, Rohatgi R, Ma L, Kirschner MW. 2001. CR16 forms a complex with N-WASP in brain and is a novel member of a conserved proline-rich actin-binding protein family. *Proc. Natl. Acad. Sci. USA* 98:11306–11

Hordijk PL, ten Klooster JP, van der Kammen RA, Michiels F, Oomen LC, Collard JG. 1997. Inhibition of invasion of epithelial cells by Tiam1-Rac signaling. *Science* 278:1464–66

Hou XS, Goldstein ES, Perrimon N. 1997. *Drosophila* Jun relays the Jun amino-terminal kinase signal transduction pathway to the Decapentaplegic signal transduction pathway in regulating epithelial cell sheet movement. *Genes Dev.* 11:1728–37

Houssa B, de Widt J, Kranenburg O, Moolenaar WH, van Blitterswijk WJ. 1999. Diacyl-glycerol kinase theta binds to and is negatively regulated by active RhoA. *J. Biol. Chem.* 274:6820–22

Hu W, Bellone CJ, Baldassare JJ. 1999. RhoA stimulates p27(Kip) degradation through its regulation of cyclin E/CDK2 activity. *J. Biol. Chem.* 274:3396–401

Huang C, Rajfur Z, Borchers C, Schaller MD, Jacobson K. 2003. JNK phosphorylates paxillin and regulates cell migration. *Nature* 424:219–23

Illenberger D, Schwald F, Pimmer D, Binder W, Maier G, et al. 1998. Stimulation of phospholipase C-beta2 by the Rho GTPases Cdc42Hs and Rac1. *EMBO J.* 17:6241–49

Innocenti M, Zucconi A, Disanza A, Frittoli E, Areces LB, et al. 2004. Abi1 is essential for the formation and activation of a WAVE2 signalling complex. *Nat. Cell Biol.* 6:319–27

Irie K, Shimizu K, Sakisaka T, Ikeda W, Takai Y. 2004. Roles and modes of action of nectins in cell-cell adhesion. *Semin. Cell Dev. Biol.* 15:643–56

Itoh M, Sasaki H, Furuse M, Ozaki H, Kita T, Tsukita S. 2001. Junctional adhesion molecule (JAM) binds to PAR-3: a possible mechanism for the recruitment of PAR-3 to tight junctions. *J. Cell Biol.* 154:491–97

Itoh RE, Kurokawa K, Ohba Y, Yoshizaki H, Mochizuki N, Matsuda M. 2002. Activation of rac and cdc42 video imaged by fluorescent resonance energy transfer-based single-molecule probes in the membrane of living cells. *Mol. Cell Biol.* 22:6582–91

Izumi Y, Hirose T, Tamai Y, Hirai S, Nagashima Y, et al. 1998. An atypical PKC directly associates and colocalizes at the epithelial tight junction with ASIP, a mammalian homologue of *Caenorhabditis elegans* polarity protein PAR-3. *J. Cell Biol.* 143:95–106

Jacinto A, Wood W, Balayo T, Turmaine M, Martinez-Arias A, Martin P. 2000. Dynamic actin-based epithelial adhesion and cell matching during *Drosophila* dorsal closure. *Curr. Biol.* 10:1420–26

Jaffe AB, Hall A. 2002. Rho GTPases in transformation and metastasis. *Adv. Cancer Res.* 84:57–80

Jaffe AB, Hall A, Schmidt A. 2005. Association of CNK1 with Rho guanine nucleotide exchange factors controls signaling specificity downstream of Rho. *Curr. Biol.* 15:405–12

Jiang H, Guo W, Liang X, Rao Y. 2005. Both the establishment and the maintenance of neuronal polarity require active mechanisms: critical roles of GSK-3beta and its upstream regulators. *Cell* 120:123–35

Joneson T, Bar-Sagi D. 1998. A Rac1 effector site controlling mitogenesis through superoxide production. *J. Biol. Chem.* 273:17991–94

Joyce D, Bouzahzah B, Fu M, Albanese C, D'Amico M, et al. 1999. Integration of Rac-dependent regulation of cyclin D1 transcription through a nuclear factor-kappaB-dependent pathway. *J. Biol. Chem.* 274:25245–49

Kato T, Watanabe N, Morishima Y, Fujita A, Ishizaki T, Narumiya S. 2001. Localization of a mammalian homolog of diaphanous, mDia1, to the mitotic spindle in HeLa cells. *J. Cell Sci.* 114:775–84

Kawakatsu T, Shimizu K, Honda T, Fukuhara T, Hoshino T, Takai Y. 2002. *Trans*-interactions of nectins induce formation of filopodia and lamellipodia through the respective activation of Cdc42 and Rac small G proteins. *J. Biol. Chem.* 277:50749–55

Kheradmand F, Werner E, Tremble P, Symons M, Werb Z. 1998. Role of Rac1 and oxygen radicals in collagenase-1 expression induced by cell shape change. *Science* 280:898–902

Komatsu S, Yano T, Shibata M, Tuft RA, Ikebe M. 2000. Effects of the regulatory light chain phosphorylation of myosin II on mitosis and cytokinesis of mammalian cells. *J. Biol. Chem.* 275:34512–20

Kosako H, Yoshida T, Matsumura F, Ishizaki T, Narumiya S, Inagaki M. 2000. Rho-kinase/ROCK is involved in cytokinesis through the phosphorylation of myosin light chain and not ezrin/radixin/moesin proteins at the cleavage furrow. *Oncogene* 19:6059–64

Kraynov VS, Chamberlain C, Bokoch GM, Schwartz MA, Slabaugh S, Hahn KM. 2000. Localized Rac activation dynamics visualized in living cells. *Science* 290:333–37

Kroschewski R, Hall A, Mellman I. 1999. Cdc42 controls secretory and endocytic transport to the basolateral plasma membrane of MDCK cells. *Nat. Cell Biol.* 1:8–13

Kuroda S, Fukata M, Nakagawa M, Kaibuchi K. 1999. Cdc42, Rac1, and their effector IQ-GAP1 as molecular switches for cadherin-mediated cell-cell adhesion. *Biochem. Biophys. Res. Commun.* 262:1–6

Lai JM, Wu S, Huang DY, Chang ZF. 2002. Cytosolic retention of phosphorylated extracellular signal-regulated kinase and a Rho-associated kinase-mediated signal impair expression of p21(Cip1/Waf1) in phorbol 12-myristate-13-acetate-induced apoptotic cells. *Mol. Cell Biol.* 22:7581–92

Lambeth JD. 2002. Nox/Duox family of nicotinamide adenine dinucleotide (phosphate) oxidases. *Curr. Opin. Hematol.* 9:11–17

Lang P, Gesbert F, Delespine-Carmagnat M, Stancou R, Pouchelet M, Bertoglio J. 1996. Protein kinase A phosphorylation of RhoA mediates the morphological and functional effects of cyclic AMP in cytotoxic lymphocytes. *EMBO J.* 15:510–19

Lee JS, Kamijo K, Ohara N, Kitamura T, Miki T. 2004. MgcRacGAP regulates cortical activity through RhoA during cytokinesis. *Exp. Cell Res.* 293:275–82

Li Z, Hannigan M, Mo Z, Liu B, Lu W, et al. 2003. Directional sensing requires G beta gamma-mediated PAK1 and PIX alpha-dependent activation of Cdc42. *Cell* 114:215–27

Lin D, Edwards AS, Fawcett JP, Mbamalu G, Scott JD, Pawson T. 2000. A mammalian PAR-3-PAR-6 complex implicated in Cdc42/Rac1 and aPKC signalling and cell polarity. *Nat. Cell Biol.* 2:540–47

Madaule P, Eda M, Watanabe N, Fujisawa K, Matsuoka T, et al. 1998. Role of citron kinase as a target of the small GTPase Rho in cytokinesis. *Nature* 394:491–4

Magie CR, Pinto-Santini D, Parkhurst SM. 2002. Rho1 interacts with p120ctn and alpha-catenin, and regulates cadherin-based adherens junction components in *Drosophila*. *Development* 129:3771–82

Malliri A, van Es S, Huveneers S, Collard JG. 2004. The Rac exchange factor Tiam1 is required for the establishment and maintenance of cadherin-based adhesions. *J. Biol. Chem.* 279:30092–98

Martinez-Quiles N, Rohatgi R, Anton IM, Medina M, Saville SP, et al. 2001. WIP regulates N-WASP-mediated actin polymerization and filopodium formation. *Nat. Cell Biol.* 3:484–91

Matsumura F, Ono S, Yamakita Y, Totsukawa G, Yamashiro S. 1998. Specific localization of serine 19 phosphorylated myosin II during cell locomotion and mitosis of cultured cells. *J. Cell Biol.* 140:119–29

Merlot S, Firtel RA. 2003. Leading the way: directional sensing through phosphatidylinositol 3-kinase and other signaling pathways. *J. Cell Sci.* 116:3471–18

Mettouchi A, Klein S, Guo W, Lopez-Lago M, Lemichez E, et al. 2001. Integrin-specific activation of Rac controls progression through the G_1 phase of the cell cycle. *Mol. Cell* 8:115–27

Millard TH, Sharp SJ, Machesky LM. 2004. Signalling to actin assembly via the WASP (Wiskott-Aldrich syndrome protein)-family proteins and the Arp2/3 complex. *Biochem. J.* 380:1–17

Minden A, Lin A, Claret FX, Abo A, Karin M. 1995. Selective activation of the JNK signaling cascade and c-Jun transcriptional activity by the small GTPases Rac and Cdc42Hs. *Cell* 81:1147–57

Miralles F, Posern G, Zaromytidou AI, Treisman R. 2003. Actin dynamics control SRF activity by regulation of its coactivator MAL. *Cell* 113:329–42

Morrison DK, Davis RJ. 2003. Regulation of MAP kinase signaling modules by scaffold proteins in mammals. *Annu. Rev. Cell Dev. Biol.* 19:91–118

Musch A. 2004. Microtubule organization and function in epithelial cells. *Traffic* 5:1–9

Narumiya S, Oceguera-Yanez F, Yasuda S. 2004. A new look at Rho GTPases in cell cycle: role in kinetochore-microtubule attachment. *Cell Cycle* 3:855–57

Nobes CD, Hall A. 1999. Rho GTPases control polarity, protrusion, and adhesion during cell movement. *J. Cell Biol.* 144:1235–44

Noritake J, Fukata M, Sato K, Nakagawa M, Watanabe T, et al. 2004. Positive role of IQGAP1, an effector of Rac1, in actin-meshwork formation at sites of cell-cell contact. *Mol. Biol. Cell* 15:1065–76

Oceguera-Yanez F, Kimura K, Yasuda S, Higashida C, Kitamura T, et al. 2005. Ect2 and MgcRacGAP regulate the activation and function of Cdc42 in mitosis. *J. Cell Biol.* 168:221–32

Ohashi K, Nagata K, Maekawa M, Ishizaki T, Narumiya S, Mizuno K. 2000. Rho-associated kinase ROCK activates LIM-kinase 1 by phosphorylation at threonine 508 within the activation loop. *J. Biol. Chem.* 275:3577–82

Olofsson B. 1999. Rho guanine dissociation inhibitors: pivotal molecules in cellular signalling. *Cell Signal* 11:545–54

Olson MF, Ashworth A, Hall A. 1995. An essential role for Rho, Rac, and Cdc42 GTPases in cell cycle progression through G1. *Science* 269:1270–72

Olson MF, Paterson HF, Marshall CJ. 1998. Signals from Ras and Rho GTPases interact to regulate expression of p21Waf1/Cip1. *Nature* 394:295–99

Palazzo AF, Cook TA, Alberts AS, Gundersen GG. 2001. mDia mediates Rho-regulated formation and orientation of stable microtubules. *Nat. Cell Biol.* 3:723–29

Perona R, Montaner S, Saniger L, Sanchez-Perez I, Bravo R, Lacal JC. 1997. Activation of the nuclear factor-kappaB by Rho, CDC42, and Rac-1 proteins. *Genes Dev.* 11:463–75

Peterson FC, Penkert RR, Volkman BF, Prehoda KE. 2004. Cdc42 regulates the Par-6 PDZ domain through an allosteric CRIB-PDZ transition. *Mol. Cell* 13:665–76

Plant PJ, Fawcett JP, Lin DC, Holdorf AD, Binns K, et al. 2003. A polarity complex of mPar-6 and atypical PKC binds, phosphorylates and regulates mammalian Lgl. *Nat. Cell Biol.* 5:301–8

Pollard TD, Borisy GG. 2003. Cellular motility driven by assembly and disassembly of actin filaments. *Cell* 112:453–65

Prokopenko SN, Brumby A, O'Keefe L, Prior L, He Y, et al. 1999. A putative exchange factor for Rho1 GTPase is required for initiation of cytokinesis in *Drosophila*. *Genes Dev.* 13:2301–14

Puls A, Eliopoulos AG, Nobes CD, Bridges T, Young LS, Hall A. 1999. Activation of the small GTPase Cdc42 by the inflammatory cytokines TNF-α and IL-1, and by the Epstein-Barr virus transforming protein LMP1. *J. Cell Sci.* 112:2983–92

Ricos MG, Harden N, Sem KP, Lim L, Chia W. 1999. Dcdc42 acts in TGF-beta signaling during *Drosophila* morphogenesis: distinct roles for the Drac1/JNK and Dcdc42/TGF-beta cascades in cytoskeletal regulation. *J. Cell Sci.* 112(Pt 8):1225–35

Ridley AJ, Schwartz MA, Burridge K, Firtel RA, Ginsberg MH, et al. 2003. Cell migration: integrating signals from front to back. *Science* 302:1704–9

Riento K, Ridley AJ. 2003. Rocks: multifunctional kinases in cell behaviour. *Nat. Rev. Mol. Cell Biol.* 4:446–56

Roovers K, Assoian RK. 2003. Effects of rho kinase and actin stress fibers on sustained extracellular signal-regulated kinase activity and activation of G_1 phase cyclin-dependent kinases. *Mol. Cell Biol.* 23:4283–94

Roovers K, Klein EA, Castagnino P, Assoian RK. 2003. Nuclear translocation of LIM kinase mediates Rho-Rho kinase regulation of cyclin D1 expression. *Dev. Cell* 5:273–84

Rosenblatt J, Cramer LP, Baum B, McGee KM. 2004. Myosin II-dependent cortical movement is required for centrosome separation and positioning during mitotic spindle assembly. *Cell* 117:361–72

Sahai E, Marshall CJ. 2003. Differing modes of tumour cell invasion have distinct requirements for Rho/ROCK signalling and extracellular proteolysis. *Nat. Cell Biol.* 5:711–19

Sahai E, Olson MF, Marshall CJ. 2001. Cross-talk between Ras and Rho signalling pathways in transformation favours proliferation and increased motility. *EMBO J.* 20:755–66

Sander EE, van Delft S, ten Klooster JP, Reid T, van der Kammen RA, et al. 1998. Matrix-dependent Tiam1/Rac signaling in epithelial cells promotes either cell-cell adhesion or cell migration and is regulated by phosphatidylinositol 3-kinase. *J. Cell Biol.* 143:1385–98

Schmidt A, Hall A. 2002. Guanine nucleotide exchange factors for Rho GTPases: turning on the switch. *Genes Dev.* 16:1587–609

Schwamborn JC, Puschel AW. 2004. The sequential activity of the GTPases Rap1B and Cdc42 determines neuronal polarity. *Nat. Neurosci.* 7:923–29

Shi SH, Cheng T, Jan LY, Jan YN. 2004. APC and GSK-3beta are involved in mPar3 targeting to the nascent axon and establishment of neuronal polarity. *Curr. Biol.* 14:2025–32

Shi SH, Jan LY, Jan YN. 2003. Hippocampal neuronal polarity specified by spatially localized mPar3/mPar6 and PI 3-kinase activity. *Cell* 112:63–75

Simon MN, De Virgilio C, Souza B, Pringle JR, Abo A, Reed SI. 1995. Role for the Rho-family GTPase Cdc42 in yeast mating-pheromone signal pathway. *Nature* 376:702–5

Sugihara K, Asano S, Tanaka K, Iwamatsu A, Okawa K, Ohta Y. 2002. The exocyst complex binds the small GTPase RalA to mediate filopodia formation. *Nat. Cell Biol.* 4:73–78

Suzuki A, Yamanaka T, Hirose T, Manabe N, Mizuno K, et al. 2001. Atypical protein kinase C is involved in the evolutionarily conserved par protein complex and plays a critical role in establishing epithelia-specific junctional structures. *J. Cell Biol.* 152:1183–96

Svitkina TM, Borisy GG. 1999. Arp2/3 complex and actin depolymerizing factor/cofilin in dendritic organization and treadmilling of actin filament array in lamellipodia. *J. Cell Biol.* 145:1009–26

Svitkina TM, Bulanova EA, Chaga OY, Vignjevic DM, Kojima S, et al. 2003. Mechanism of filopodia initiation by reorganization of a dendritic network. *J. Cell Biol.* 160:409–21

Takaishi K, Sasaki T, Kotani H, Nishioka H, Takai Y. 1997. Regulation of cell-cell adhesion by rac and rho small G proteins in MDCK cells. *J. Cell Biol.* 139:1047–59

Takeya R, Sumimoto H. 2003. Molecular mechanism for activation of superoxide-producing NADPH oxidases. *Mol. Cell.* 16:271–77

Takeya R, Ueno N, Kami K, Taura M, Kohjima M, et al. 2003. Novel human homologues of p47phox and p67phox participate in activation of superoxide-producing NADPH oxidases. *J. Biol. Chem.* 278:25234–46

Tapon N, Nagata K, Lamarche N, Hall A. 1998. A new rac target POSH is an SH3-containing scaffold protein involved in the JNK and NF-kappaB signalling pathways. *EMBO J.* 17:1395–404

Tatsumoto T, Xie X, Blumenthal R, Okamoto I, Miki T. 1999. Human ECT2 is an exchange factor for Rho GTPases, phosphorylated in G2/M phases, and involved in cytokinesis. *J. Cell Biol.* 147:921–28

Teramoto H, Coso OA, Miyata H, Igishi T, Miki T, Gutkind JS. 1996. Signaling from the small GTP-binding proteins Rac1 and Cdc42 to the c-Jun N-terminal kinase/stress-activated protein kinase pathway. A role for mixed lineage kinase 3/protein-tyrosine kinase 1, a novel member of the mixed lineage kinase family. *J. Biol. Chem.* 271:27225–28

Tolias KF, Hartwig JH, Ishihara H, Shibasaki Y, Cantley LC, Carpenter CL. 2000. Type Iα phosphatidylinositol-4-phosphate 5-kinase mediates Rac-dependent actin assembly. *Curr. Biol.* 10:153–56

Vaezi A, Bauer C, Vasioukhin V, Fuchs E. 2002. Actin cable dynamics and Rho/Rock orchestrate a polarized cytoskeletal architecture in the early steps of assembling a stratified epithelium. *Dev. Cell* 3:367–81

Vasioukhin V, Bauer C, Yin M, Fuchs E. 2000. Directed actin polymerization is the driving force for epithelial cell-cell adhesion. *Cell* 100:209–19

Vidal A, Millard SS, Miller JP, Koff A. 2002. Rho activity can alter the translation of p27 mRNA and is important for RasV12-induced transformation in a manner dependent on p27 status. *J. Biol. Chem.* 277:16433–40

Vignjevic D, Yarar D, Welch MD, Peloquin J, Svitkina T, Borisy GG. 2003. Formation of filopodia-like bundles in vitro from a dendritic network. *J. Cell Biol.* 160:951–62

Wang F, Herzmark P, Weiner OD, Srinivasan S, Servant G, Bourne HR. 2002. Lipid products of PI$_3$Ks maintain persistent cell polarity and directed motility in neutrophils. *Nat. Cell Biol.* 4:513–8

Wang HR, Zhang Y, Ozdamar B, Ogunjimi AA, Alexandrova E, et al. 2003. Regulation of cell polarity and protrusion formation by targeting RhoA for degradation. *Science* 302:1775–79

Watanabe T, Wang S, Noritake J, Sato K, Fukata M, et al. 2004. Interaction with IQGAP1 links APC to Rac1, Cdc42, and actin filaments during cell polarization and migration. *Dev. Cell* 7:871–83

Weber JD, Hu W, Jefcoat SC Jr, Raben DM, Baldassare JJ. 1997. Ras-stimulated extracellular signal-related kinase 1 and RhoA activities coordinate platelet-derived growth factor-induced G1 progression through the independent regulation of cyclin D1 and p27. *J. Biol. Chem.* 272:32966–71

Weernink PA, Meletiadis K, Hommeltenberg S, Hinz M, Ishihara H, et al. 2004. Activation of type I phosphatidylinositol 4-phosphate 5-kinase isoforms by the Rho GTPases, RhoA, Rac1, and Cdc42. *J. Biol. Chem.* 279:7840–49

Welsh CF, Roovers K, Villanueva J, Liu Y, Schwartz MA, Assoian RK. 2001. Timing of cyclin D1 expression within G1 phase is controlled by Rho. *Nat. Cell Biol.* 3:950–57

Wen Y, Eng CH, Schmoranzer J, Cabrera-Poch N, Morris EJ, et al. 2004. EB1 and APC bind to mDia to stabilize microtubules downstream of Rho and promote cell migration. *Nat. Cell Biol.* 6:820–30

Westwick JK, Lambert QT, Clark GJ, Symons M, Van Aelst L, et al. 1997. Rac regulation of transformation, gene expression, and actin organization by multiple, PAK-independent pathways. *Mol. Cell Biol.* 17:1324–35

Wittmann T, Waterman-Storer CM. 2001. Cell motility: Can Rho GTPases and microtubules point the way? *J. Cell Sci.* 114:3795–803

Worthylake RA, Burridge K. 2003. RhoA and ROCK promote migration by limiting membrane protrusions. *J. Biol. Chem.* 278:13578–84

Xu Z, Kukekov NV, Greene LA. 2003. POSH acts as a scaffold for a multiprotein complex that mediates JNK activation in apoptosis. *EMBO J.* 22:252–61

Yamamoto M, Marui N, Sakai T, Morii N, Kozaki S, et al. 1993. ADP-ribosylation of the rhoA gene product by botulinum C3 exoenzyme causes Swiss 3T3 cells to accumulate in the G1 phase of the cell cycle. *Oncogene* 8:1449–55

Yamanaka T, Horikoshi Y, Sugiyama Y, Ishiyama C, Suzuki A, et al. 2003. Mammalian Lgl forms a protein complex with PAR-6 and aPKC independently of PAR-3 to regulate epithelial cell polarity. *Curr. Biol.* 13:734–43

Yamanaka T, Horikoshi Y, Suzuki A, Sugiyama Y, Kitamura K, et al. 2001. PAR-6 regulates aPKC activity in a novel way and mediates cell-cell contact-induced formation of the epithelial junctional complex. *Genes Cells* 6:721–31

Yamashiro S, Totsukawa G, Yamakita Y, Sasaki Y, Madaule P, et al. 2003. Citron kinase, a Rho-dependent kinase, induces di-phosphorylation of regulatory light chain of myosin II. *Mol. Biol. Cell* 14:1745–56

Yasuda S, Oceguera-Yanez F, Kato T, Okamoto M, Yonemura S, et al. 2004. Cdc42 and mDia3 regulate microtubule attachment to kinetochores. *Nature* 428:767–71

Yoshimura T, Kawano Y, Arimura N, Kawabata S, Kikuchi A, Kaibuchi K. 2005. GSK-3beta regulates phosphorylation of CRMP-2 and neuronal polarity. *Cell* 120:137–49

Yu W, Datta A, Leroy P, O'Brien LE, Mak G, et al. 2005. β1-integrin orients epithelial polarity via Rac1 and laminin. *Mol. Biol. Cell* 16:433–45

Zegers MM, O'Brien LE, Yu W, Datta A, Mostov KE. 2003. Epithelial polarity and tubulogenesis in vitro. *Trends Cell Biol.* 13:169–76

Zhang X, Bi E, Novick P, Du L, Kozminski KG, et al. 2001. Cdc42 interacts with the exocyst and regulates polarized secretion. *J. Biol. Chem.* 276:46745–50

Zheng Y, Bagrodia S, Cerione RA. 1994. Activation of phosphoinositide 3-kinase activity by Cdc42Hs binding to p85. *J. Biol. Chem.* 269:18727–30

Zigmond SH. 2004. Formin-induced nucleation of actin filaments. *Curr. Opin. Cell Biol.* 16:99–105

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Contents

INDEXES

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